2887-Pos Board B579
Extracellular Matrix Elasticity Determines Stem Cell Fate through Stretch-Activated Ion Channels
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Extracellular matrix elasticity is a well-established determinant of lineage specification for many stem cell types. In neural stem cells, substrate stiffness controls glial versus neu-ronal specification. While matrix elasticity is known to be transduced at focal adhesion zones, the molecular mechanisms that direct mechanosensitive fate pathways are not well understood. Here we use a combination of electrophysiology, live cell microscopy and mo-lecular techniques to uncover a missing player in mechanosensitive lineage commitment. We find that human neural stem/progenitor cells (hNSPCs) express stretch-activated ion channels (SACs), whose activity triggers spontaneous, transient Ca^{2+} signals that are modu-lated by substrate stiffness. Pharmacological inhibition of SAC activity suppresses neuronal differentiation while promoting astrocyte formation, indicating that SACs are important for lineage choice. We will present results from an RNAi approach aimed at determining the molecular identity of the hNSPC SAC channel.

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Effect of General Anesthetics and Alcohols on Prestin (SLC26A5) Function
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Prestin is a membrane protein essential to the electromotility of outer-hair cells (OHC) in the cochlea. This protein with piezoelectric properties has shown sensitivity to changes in cell membrane physical properties. The presence of molecules which impact the lateral pressure or the fluidity of the membrane -such as cholesterol, NSAIDs or lipophilic ions- can alter the electrophysiological properties of prestin as well as the electromotility. In an OHC, the nonlinear capacitance (NLC) and the electromotility are both conferred by prestin and coupled. This allows monitoring the NLC as a surrogate measure of prestin's function.
Here we describe the impact of general anesthetics and alcohols on prestin, and aim to use this protein as a model system for studying membrane-protein interaction through changes in the NLC. We tested the effect on prestin of alcohols with various C-chains (C2 to C10) and some general anesthetics (GA: propofol, isoflurane, halothane, chloroform, etomidate and xylazine). All the alcohols tested trigger a dose-dependent shift in the characteristic voltage at half-maximal charge transfer (V1/2), with the sensitivity increasing as the alcohols' carbon-chain is longer. The direction of the shift changes with concentration, the lower concentration cause a negative shift while higher concentrations shift V1/2 closer to 0mV. These shifts are correlated with changes in the linear capacitance of the cell. All of the GA we tested caused a dose-dependent increase in charge density at sub-millimolar concentrations as well as a shift of V1/2 toward hyperpolarized voltages. The sensitivity of prestin to these molecules seems to follow the Meyer/Overton correlation.
The shift in the NLC as well as the increase in charge density can modify the function of the OHC at resting potential and alter cochlear amplification.

2889-Pos Board B581
Theoretical and Experimental Framework of Neurite Response to Chemical Gradients in 3D Matrices
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During the development of nervous system, various attractive and repulsive signals in the surrounding extracellular matrix (ECM) environment guide the growing neurites along specific directions to reach their intended targets. Neuronal motility is controlled by extracellular signal-sensing via the growth cone at the neurite tip, including chemoattractive and repulsive cues. We quantitatively investigate this response using a combination of mathematical modeling and in vitro experiments, and determine the role of guidance cues and ECM on neurite outgrowth and turning. A microfluidic system was used to show that cortical neurite outgrowth and turning under chemogradients (IGF-1 or BDNF) within 3D scaffolds is highly regulated by the source concentration of the guidance cue and the physical characteristics of the scaffold. A partial differential equation model of neurite outgrowth has been proposed that may be used as a predictive tool. The parameters for the chemotaxis term in the model are determined from experimental data. Resulting model simulations demonstrate how neurite outgrowth was critically influenced by the experimental variables, which was further supported by experimental data on cell-surface-receptor expressions. We demonstrate that our model results are in excellent agreement with experimental findings.

2890-Pos Board B582
Single-Molecule Analysis of LFA-1/ICAM-1 Binding in Lymphocyte
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Leukocyte integrin LFA-1 is activated through inside-out signaling by Rap1 upon chemokineand TCR stimulation, thereby regulating lymphocyte migration and arrest via immunological synapse (IS) formation However, the precise mechanisms of the spacio-temporal regulation of LFA-1 binding to ICAM-1 and the role of Rap1 signaling during these processes are not well understood. To address this issue, we established live imaging of LFA-1 and ICAM-1 at the single-molecule levels on supported planner lipid bilayer to measure distribution and binding kinetics of LFA-1 and ICAM-1. To visualize LFA-1 we attached halotag to LFA-1 tail. We also labeled ICAM-1 using TMR derived dyes at low concentrations and incorporated into lipid bilayers. LFA-1 and ICAM-1 was visualized and tracked at the single-molecule level using TIRFM. In chemokine-stimulated T cell migration, randomly diffusing ICAM-1 was frequently captured in attached area and were relatively mobile with short life-time. We set up the conditions of the immunological synapse formation of OT-II T cells on supported planar membrane presenting OVA-peptide MHC with ICAM-1. We confirmed that primary OT-II T cells exhibited typical IS with clear cSMAC formed by TCR/pMHC surround by pSMAC formed by LFA-1/ICAM-1. The single-molecule analysis revealed that long-lived immobilized ICAM-1 in the distinct areas of pSMAC. In contrast, OT-II T cells deficient for Rap1 effector Mst1 exhibited inefficient attachment with aberrant IS. The immobilized ICAM-1 populations were also diminished. These results showed the binding kinetics of LFA-1 and ICAM-1 was distinct between chemokine and TCR and involvement of Mst1 ininside-out signaling leading to immunological synapse. Further studies are currently under way to examine the role of LFA-1 tails and Rap1 signaling.

2891-Pos Board B583
Bayesian Analysis Distinguishes Brownian Motion from Motor-Driven Transport within Organelle Trajectories
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Many organelles and vesicles move in a start-stop manner in live cells when observed by optical microscopy at 10 to 100 frames/s. One explanation for start-stop behavior is that the vesicles switch between a driven state in which they are being actively pulled by motor proteins, and a Brownian state in which they obey the laws of diffusion in the cytoplasm. To test this idea, we have carried out a hidden Markov, variational Bayesian Expectation Maximization, Gaussian mixture model ("Bayes") analysis. Either vesicle velocity v(t) or the direction of travel theta(t) was used to "train" the model. When tested with simulated tracks, Bayes reliably determined the number of states K_best corresponding to the number of distinct physical processes required to describe the data. The mean and variance of the velocity or direction were also found reliably for each state in the simulated dataset. The assignment of individual frames to particular states showed few false positives or false negatives as long as the vesicle remained in each state for 6 or more adjacent frames. Once each frame was assigned to a state by Bayes, the mean-squared displacement (msd) for each state was computed, displaying the distinctive t-dependence of its physical origin, e.g. motor-driven, Brownian, etc. Individual tracks of fluorescently labeled peroxisomes in HME cells and unlabeled vesicles in PC12 cells were analyzed in the same manner to separate driven from Brownian bouts in an objective manner.
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2892-Pos Board B584
Mismatch Repair Protein Mobility in Human Cancer Cells
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We will report our measurements on the mobility of the mismatch repair protein MSH2 in cells from a cell line that has normal, immortal, and tumorigenic cells. Additionally we have measured the mobility of the protein in metastatic

MDA-MB-231 cells. Specifically, we plan to report on the diffusion coefficients of MSH2 as measured using Raster Image Correlation Spectroscopy, a single molecule technique that takes advantage of the natural scanning nature of confocal microscopes. Results will be reported on all four cell types, with separate measurements for both cytoplasmic and nuclear cell regions. Results from this study will allow a comparison of protein diffusion in cells that are at different stages of neoplastic transformation.

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2893-Pos Board B585

Chromatin Mechanically Buffers Cytoskeletal Forces at the Nuclear Envelope

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Building tissues from individual cells requires mechanisms to communicate mechanical properties between cells and organelles, as well as the ability to alter the physical properties of cellular materials. The LINC (**Li**nker of **N**ucleoskeleton and **C**ytoskeleton) complex, which bridges both membranes of the nuclear envelope, may provide one such mechanism. Cytoskeletal forces are delivered onto the nucleus via LINC complexes, which physically couple the cytoplasmic cytoskeleton to the nuclear interior and its associated chromatin. These complexes can mechanically transmit cytoskeletal forces to chromatin, suggesting the possibility that they may participate in mechanotransduction. While our previous work suggests an additional role for chromatin and its connections to the LINC complex and the nuclear envelope in maintaining nuclear integrity and buffering cytoskeletal forces, a quantitative analysis of these properties has been lacking.

We explicitly address the individual contributions of chromatin and chromatin-binding membrane proteins to nuclear integrity in the genetic organism, fission yeast (*Schizosaccharomyces pombe*). We have developed a combined optical tweezers/epifluorescence assay that allows us to manipulate, exert forces on and image isolated nuclei. Deriving nuclei from the genetically facile fission yeast model, we have interrogated how the presence of individual nuclear components and their crosslinking contributes to the ensemble physical behavior of the nucleus. Using genetic perturbations, we implicate integral inner nuclear membrane proteins that couple chromatin to the nuclear envelope in defining nuclear stiffness and supporting nuclear elasticity. The relevance of these findings to *in vivo* nuclear mechanics has been established by measuring nuclear envelope fluctuations in living *S. pombe* cells. These results provide new insights into how the mechanical properties of nuclei can be modulated. In addition, our work suggests that cytoskeletal forces coupled to the nuclear interior by the LINC complex contribute to overall chromatin mobility.

2894-Pos Board B586

Quantification of Directional Migration by a Characteristic Directionality Time

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Many cell types can bias their direction of locomotion by coupling to external cues. Characteristics such as how fast a cell migrates and the convolution of its migration path can be quantified to provide metrics that determine which biochemical and biomechanical factors affect directional cell migration, and by how much. To be useful, these metrics should be reproducible from one experimental setting to another, and the resulting quantitative analysis should describe the underlying processes that occur in the system. In this work, several metrics used to characterize cell migration, such as turning angle distribution and straightness index, are evaluated in the context of reproducibility and quantitative interpretability. We discuss how these existing metrics can be modified to be more reproducible, and introduce a new metric called directionality time that can be used to characterize convolution of the migration path. Directionality time is measured based on fitting the slope of the mean squared displacement in log-log coordinates. We apply three selected random walk models to demonstrate that the functional form of the fit equation is approximately model invariant. Directionality time is reproducible because the numeric result takes into account tracking noise and is independent of the time interval of path measurement, the two variables that tend to change from one experiment to another. The robustness of this metric is tested via computer simulation and against experiment data of neutrophils directionally migrating towards a chemoattractant.

2895-Pos Board B587

Impact of Cell Shape on Cell Migration Behavior on Elastic Substrate

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Cell shape is known to have profound effects on a number of cell behaviors. In this paper we have studied the role of cell shape in cell migration through modeling its effect on cell traction force distribution, traction force dependent stability of cell adhesion and matrix rigidity dependent traction force formation. To quantify the driving force of cell migration, a new parameter called the motility factor, that takes account of the effect of cell shape, matrix rigidity and dynamic stability of cell adhesion, is proposed. We found that the motility factor depends on the matrix rigidity in a biphasic manner, in consistence with the experimental observations of the biphasic dependence of cell migration speed on the matrix rigidity. We showed that the cell shape plays a pivotal role in the cell migration behavior by regulating the traction force at the cell front and rear. The larger the cell polarity, the larger the motility factor is. The keratocyte-like shape has a larger motility factor than the fibroblast-like shape, which explains why keratocyte has a much higher migration speed. The motility factor might be an appropriate parameter for a quantitative description of the driving force of cell migration.

2896-Pos Board B588

Two-Component Dissipative Particle Dynamics Model of Red Blood Cells

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By modeling the lipid bilayer and the cytoskeleton separately, we applied a two-component Dissipative Particle Dynamics (DPD) red blood cell (RBC) model to simulate several in vitro and ex-vivo experiments. First, we validated our model by comparing simulation results with the experimental measurements in micropipette aspiration, membrane fluctuations, tank treading motions and bilayer tethering in a channel flow. We explored the effects of bilayer-cytoskeletal interaction properties such as elastic stiffness, viscous frictions and strength on these experiments and resolved several controversies on RBC mechanics in the literature. In addition, we simulated the ex-vivo perfusion of healthy and malaria-infected RBCs in human spleen by modeling the RBCs passing the inter-endothelial slits, and the predicted retention rates match the experimental measurement well. We also carried out systematic parametric studies on the critical conditions for RBCs to pass through the splenic slit. Our simulation results provide a comprehensive computational framework for understanding the roles of human spleen related to RBC-borne diseases such as malaria and hereditary spherocytosis.

2897-Pos Board B589

Insights into Cytoplasmic Rheology Gained from Modeling Cellular Blebbing

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Blebbing occurs when the cytoskeleton detaches from the cell membrane, resulting in the pressure-driven flow of cytosol towards the area of detachment and local expansion of the cell membrane. Recent experiments involving blebbing cells have led to conflicting hypotheses regarding the timescale of intracellular pressure propagation. The interpretation of one set of experiments supports a poroelastic cytoplasmic model which leads to slow pressure equilibration when compared to the timescale of bleb expansion. A different study concludes that pressure equilibrates faster than the timescale of bleb expansion. To address this, a dynamic computational model of the cell was developed that includes mechanics of and the interactions between the intracellular fluid, the actin cortex, the cell membrane, and the cytoskeleton. The model results quantify the relative importance of cytoskeletal elasticity and drag in bleb expansion dynamics. This study also shows that recent multi-bleb experimental results can be explained by the combination of cytoskeletal poroelasticity with either dynamic membrane-cortex adhesion or cortical reformation.